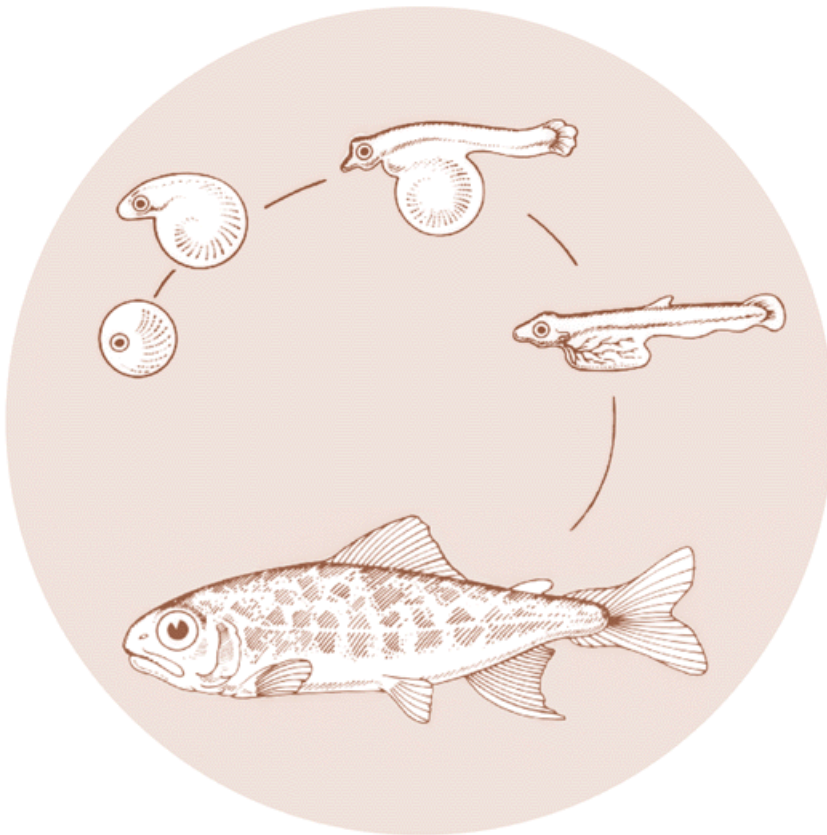


August 1992

AUGMENTED FISH HEALTH MONITORING

Completion Report



DOE/BP-35585-4



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AUGMENTED FISH HEALTH MONITORING

Completion Report

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Project No. 87-119
Contract No. DE-AI79-87BP35585

August 1992

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Introduction

The Augmented Fish Health Monitoring Project (Project) had its origin, in the mid-1980's, in perceived differences or inconsistencies in fish disease detection, diagnosis and control capabilities between the five conservation agencies rearing and releasing anadromous salmonids for fishery resource management and mitigation purposes in the Columbia River basin. Agency fish health programs varied greatly. Some agencies had personnel, equipment and funding to frequently monitor the health status of both juvenile production fish and adult salmon or steelhead trout at the time of spawning. Other agencies had much smaller programs and limited resources. These differences became better understood when the Pacific Northwest Fish Health Protection Committee developed its Model Fish Health Protection Program including recommendations for standard fish disease detection procedures. Even though some agencies could not immediately attain the goals set by the Model Program it was unanimously adopted as a desirable objective. Shortly thereafter, a multi-party planning group was assembled to help the Bonneville Power Administration (BPA) find ways to improve agency fish health programs and implement measures under the Fish and Wildlife Program of the Northwest Power Planning Council. The planning group assessed existing agency fish health monitoring capabilities, agreed upon satisfactory levels of capability to detect and identify important fish pathogens, and designed a five-year project establishing comparable fish health monitoring capability in each agency. It was strongly believed that such a project would improve the health and quality of the millions of hatchery fish released annually in the Columbia River basin and improve interagency communications and disease control coordination.

During 1986 and 1987 BPA individually negotiated five separate contracts with the fishery agencies to standardize fish health monitoring, develop a common data collection and reporting format, evaluate and monitor hatchery water supplies, improve communications and coordination, and provide a mechanism for periodic review. The

contract with the U. S. Fish and Wildlife Service was approved on May 31, 1987, the last of the five inter-agency agreements to be signed. This did not delay Service participation with the other agencies because the Service already had many elements of the Project in place under its existing Service-wide fish health policy and implementation plan,

The Service fully participated in the Project from mid-1987 through mid-1992. This completion report describes some of the many accomplishments under each of the objectives, benefits accruing from the Project, elements to be sustained by the Service after the Project ended, and Project that were not completed or that pointed out new work that needs to be done.

Description of Project Area

The Service operates 13 National Fish Hatcheries (NFH) in the Columbia River basin. Figure 1. illustrates the location of these facilities. Both the Abernathy Salmon Culture Technical Center and the Eagle Creek NFH are on tributaries to the lower Columbia River below Bonneville Dam. The Dworshak, Kooskia and Hagerman NFH's are on the Snake River system in Idaho. Leavenworth, Entiat and Winthrop NFH's are located in central Washington near the upper limits of Columbia River migration for adult anadromous fish. The remainder of the facilities are above Bonneville Dam on tributaries to the lower Columbia River. With the exception of the Spring Creek and Hagerman NFH's, all are supplied, all or in part, with surface water for at least a portion of the annual juvenile rearing cycle.

Rearing programs at the Columbia River basin NFH's (Table 1.) focus on production of anadromous fish to meet mitigation obligations for large federal water projects (dams). The Idaho facilities primarily produce summer steelhead trout but spring chinook salmon also are produced at Dworshak and Kooskia. With the exception of Spring Creek, which produces Yule" fall chinook, the Washington and Oregon facilities above Bonneville Dam produce large numbers of spring chinook salmon and to a lesser extent, coho salmon and

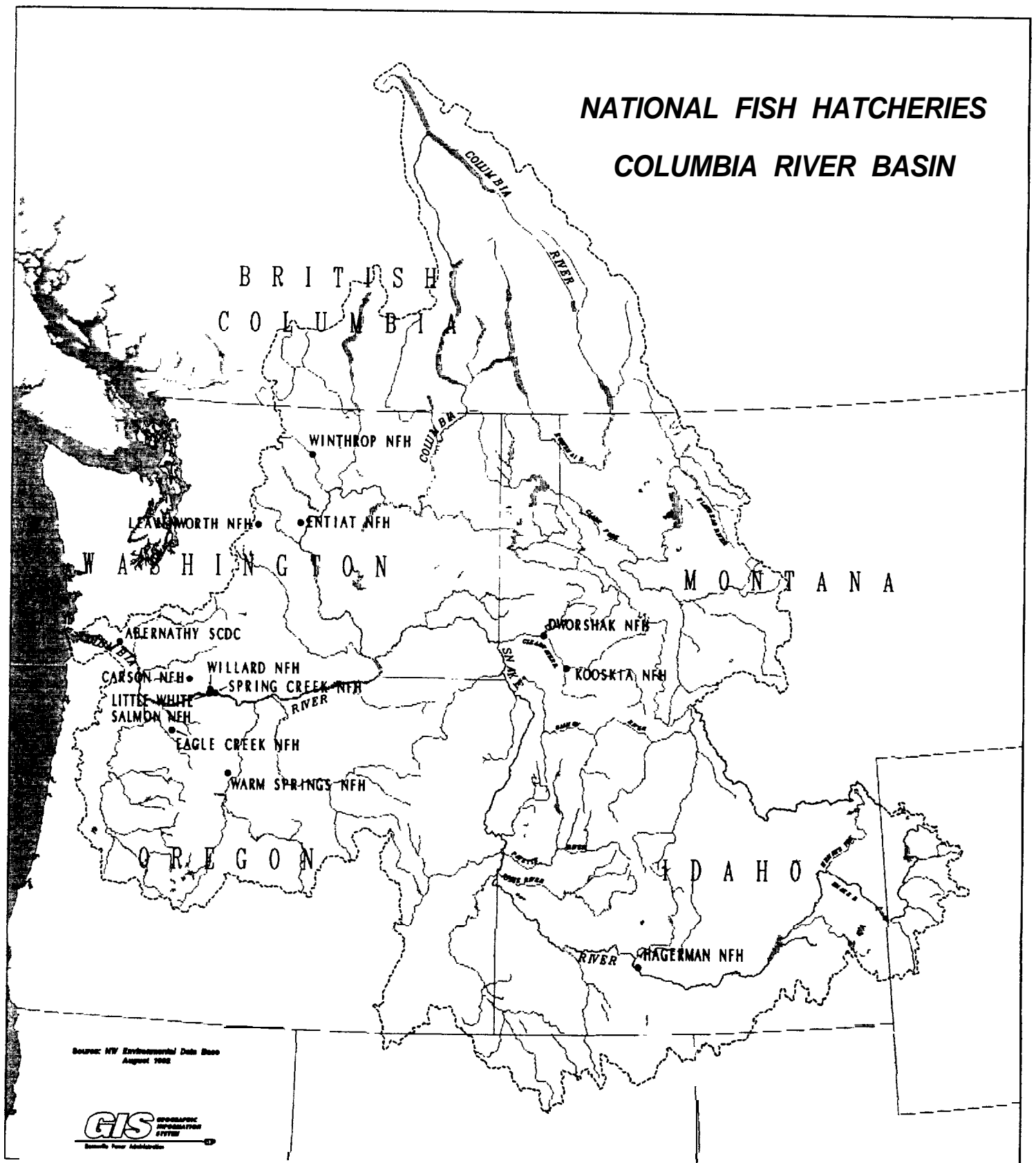


Figure 1. National Fish Hatcheries in the Columbia River basin.

other Salmonid species. The lower river facilities produce coho salmon, tule fall chinook, some chum salmon in some years, and winter steelhead trout.

Table 1. Production programs at National Fish Hatcheries in the Columbia River basin.

Facility	Location	Species reared
Abernathy SCTC	Longview, WA	Fall chinook
Carson NFH	Carson, WA	Spring chinook
Dworshak NFH	Ahsahka, ID	Spring chinook, Summer steelhead
Eagle Creek NFH	Estacada, OR	Coho, Winter steelhead
Entiat NFH	Entiat, WA	Spring chinook
Hagerman NFH	Hagerman, ID	Summer steelhead
Kooskia NFH	Kooskia, ID	Spring chinook, Summer steelhead
Leavenworth NFH	Leavenworth, WA	Spring chinook, Summer steelhead
Little White Salmon NFH	Cook, WA	Spring chinook, Fall chinook
Spring Creek NFH	Underwood, WA	Fall chinook
Spring Creek - Big White Ponds	Underwood, WA	Spring chinook
Warm Springs NFH	Warm Spring, OR	Spring chinook
Willard NFH	Willard, WA	Coho
Winthrop NFH	Winthrop, WA	Spring chinook

Project Review and Evaluation

Project design - The Project was organized under six broad objectives covering the tasks necessary to prepare for the project, conduct all required work, and to collect and analyze the data recorded during each annual rearing cycle.

The first objective dealt with obtaining the necessary personnel and equipment and making the necessary interagency arrangements to fully coordinate and carry out the project. Early planning and coordination was effected through the use of a steering committee that met quarterly to report progress, mutually discuss problems encountered, and to ensure that contract requirements were understood and were being appropriately implemented by each of the participating agencies.

Under Objective 2.0 were provisions for inter-agency communications, information transfer and the determination of “impediments to fish health” found at Columbia River basin fish hatcheries. For the latter item, each of the fisheries agencies prepared a list of specific impediments to fish health found at each facility. Such things as river water supplies introducing serious fish pathogens, inadequate water supplies, poor quality water (high temperatures in the summer, dirty water, etc.), inadequate broodstock holding and sorting facilities, inappropriate or inadequate rearing facilities, and many other factors were identified. There was, however, no mechanism for making use of the information collected in spite of the urgency noted for rectifying some of the problems identified or the long-term benefits derived.

Objective 3.0 of the Project set forth requirements for the specific technical work to accomplish standardized fish health monitoring and other required tasks at Columbia River basin fish hatcheries. This work is summarized in Table 2.

With the exception of mid-term monitoring for the prevalence of bacterial kidney disease (BKD), monitoring for the presence of erythrocytic inclusion bodies indicative of erythrocytic inclusion body syndrome (EIBS), monitoring two index stocks for organosomatic (physiological) indices, and increasing the frequency of station visits by pathologists to monthly visits, the Service was already accomplishing most Project goals before the contract for Augmented Fish Health Monitoring was negotiated. For the U. S. Fish and Wildlife Service the Project increased fish health surveillance at Columbia River NFH's by about 15 percent over pre-Project levels. In addition, the Service was

Table 2. Service technical procedures for Project implementation.

Pathogen	Host life stage	Tissue(s) sampled	Pathogen detection method
<i>Viral agents</i>			
IHNV	Juvenile	Kidney+ spleen	Cell culture: EPC ¹ and CHSE-214 ² monolayers
	Spawning adult	Kidney t spleen/ovarian fluid	" "
IPNV	All	Kidney t spleen	" "
EIBS	All	Blood film	Pinacyanol chloride stain; 2 min. exam at 1000x
<i>Bacterial agents</i>			
<i>R. salmoninatum</i>	Juvenile	Posterior kidney	FAT ³ : 50 fields at 1000x; ELISA ⁴
	Adult	Ovarian fluid	" "
<i>F. psychrophilus</i>	Juvenile	Posterior kidney	Gram stain or culture on columnaris agar
<i>A. salmonicida</i>	All	Posterior kidney	Culture on trypticase soy agar (TSA)
<i>Y. ruckeri</i>	All	Posterior kidney	" "
<i>Parasitic agents</i>			
<i>M. cerebralis</i>	Juvenile	Head cartilage	Digest method; confirm by histopathology
<i>C. shasta</i>	All	Lesion/hind gut	Light microscopy
PKX	Juvenile	Posterior kidney	Light microscopy; confirm by histopathology
<i>Monthly monitoring</i>	10 moribund juveniles	As needed	As needed
<i>Organosomatic indices</i>	60 pre-release juv.	As required	Goede, Utah D of W, Personal communication, 1987

¹ EPC = Epithelioma papillosum cyprini cells

² CHSE-214 = Chinook salmon embryo cells

³ FAT = Fluorescent antibody test

⁴ ELISA = enzyme-linked immunosorbent assay

able to establish a regional histopathology facility at the Olympia (WA) Fish Health Center to meet Service and State needs to confirm fish disease diagnoses required by the Project and for other fish health purposes.

Organosomatic indices monitoring of pre-release juvenile tule fall chinook at the Spring Creek NFH and summer steelhead trout at the Dworshak NFH also was required under Objective 3.0. This aspect of the Project was already underway at the Dworshak FHC, but was new to other Service personnel. In 1987, Ron Goede from the Utah Division of Wildlife Resources presented an inter-agency training session for technical and hatchery personnel. This training greatly facilitated the standardized sampling, assessment and recording of the numerous physiological parameters involved.

Water quantity and quality is critically important to the production of healthy fish. Objective 4.0 authorized a program to conduct studies of hatchery water supplies. A water sampling plan was jointly designed by the steering committee and submitted to BPA for approval. Unfortunately BPA did not complete contractual arrangements with an analytical laboratory to analyze the required samples. Other related environmental parameters including flow index and density index (Piper et al, 1982) were regularly reported with diagnostic data.

Objectives 5.0 and 6.0 set forth guidelines for the collection, analysis, and dissemination of the large amount of data generated by the Project. Nearly 5,000 case histories were recorded in a computer-supported database from which data abstracts were prepared and submitted quarterly. Pre-Project case histories for the years 1983, 1984, and 1985 were entered into the database from old paper records to meet requirements set for submitting Pre-Project base-line information.

Viral pathogen monitoring - Sampling and laboratory testing was performed on spawning adult Pacific salmon and steelhead trout to detect infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis (IPNV), and the erythrocytic inclusion bodies

typically associated with erythrocytic inclusion body syndrome EIBS). The Service was already monitoring for IHNV and IPNV in adult and juvenile salmonids before the Project began. The Project standardized all sampling and virological procedures, added the requirement to monitor for EIBS, and organized and standardized the reporting of results. The required virological methods (Amos, 1985) made possible the detection of other viral agents as well, including herpes virus salmonis, viral hemorrhagic septicemia virus, *Oncorhynchus masou* virus, reoviruses, and paramyxoviruses. No viruses of this latter group were detected at Columbia River basin National Fish Hatcheries during the study.

Thousands of virological samples collected from adult and juvenile salmonids spawned or reared at Columbia River basin NFH's were processed and screened each year. IHNV and EIBS were frequently encountered, usually at low levels, but occasionally serious IHN outbreaks occurred. IPNV was detected in steelhead trout in the Wenatchee River system and in rainbow trout in the upper Warm Springs River system. One of the great benefits of this Project was the collaboration that went into the development and implementation of highly sensitive and highly specific sampling and laboratory procedures for monitoring for the prevalence of fish viruses on a very large scale. In some situations, for example, at the Leavenworth NFH alone, as many as 3,500 individual adult salmon were assayed for the presence of fish viruses in a single spawning season. The information was used for the segregation of eggs from IHNV-positive adults and made it possible to identify eggs from virus-negative adults for transfer to other locations as needed for resource management purposes. Data on the incidence and prevalence of IHN also clarified the value of iodophor egg disinfection and virus-free water during incubation and early rearing in the prevention of IHN.

Bacterial pathogen monitoring - Bacterial pathogens covered by the Project included *Renibacterium salmoninarum*, the cause of bacterial kidney disease (BKD), *Flexibacter*

psychrophilus, the cause of bacterial coldwater disease (BCWD), *Aeromonas salmonicida*, the cause of furunculosis, and *Yersinia ruckeri*, the cause of enteric redmouth (ERM).

With the exception of mid-term monitoring for BKD, the Service was already exceeding Project requirements for monitoring for bacterial fish pathogens before the Project began. Mid-term monitoring for the prevalence of BKD in spring chinook juveniles had some predictive value in estimating needs for therapy later in the rearing cycle.

Figure 2. shows the prevalence of *R. salmoninarum* infections in spring chinook smolts just prior to release into the Columbia or Snake River systems for downstream migration for the years 1989 through 1992.

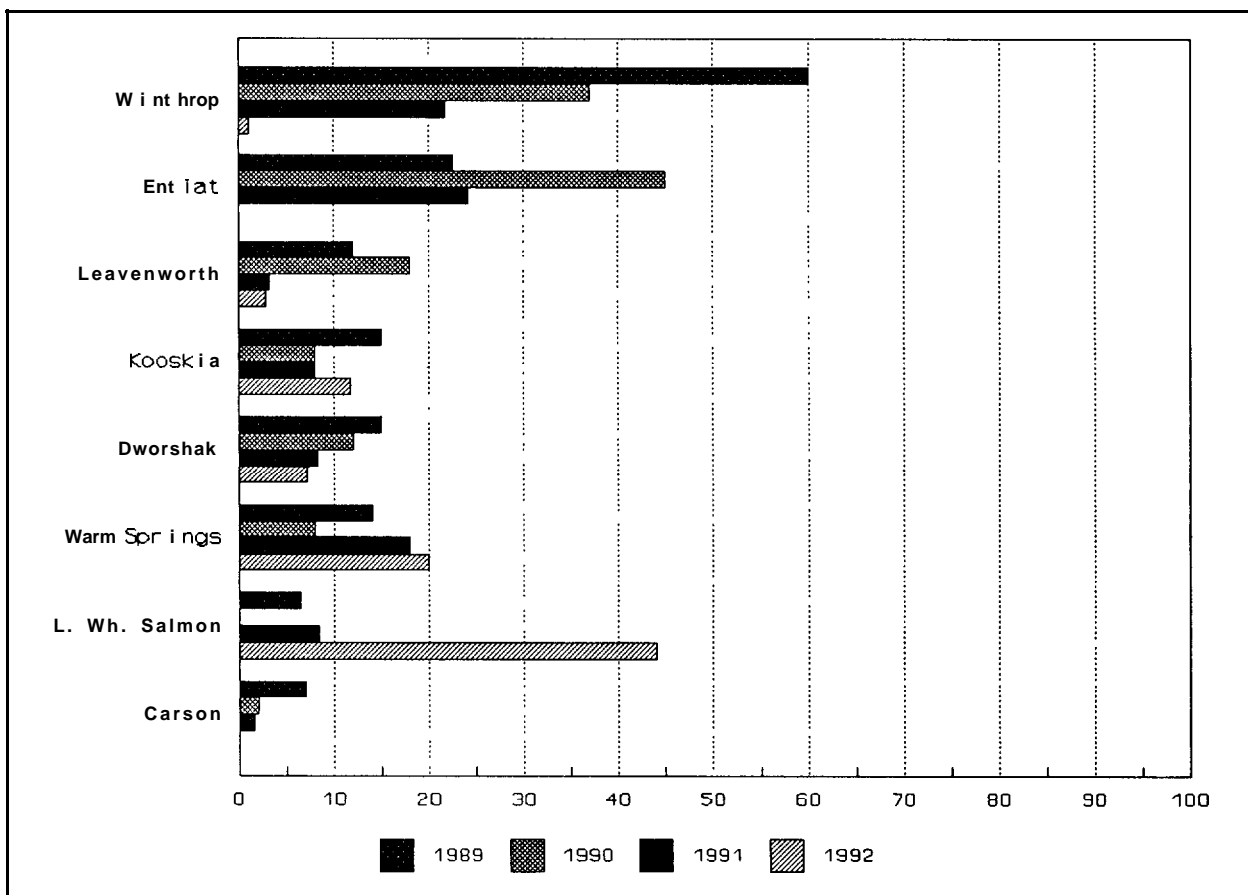


Figure 2. *K. salmoninarum* prevalence in apparently normal spring chinook smolts examined prior to release in the years 1989 through 1992.

These field studies also identified the need for a sensitive, quantitative diagnostic method. Initially, fluorescent antibody techniques (FAT) were the state-of-the-art procedure of choice for BKD monitoring. Near the end of the Project, however, highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) techniques became available for the processing of larger numbers of samples from individual adult spawners and the use of the quantitative results to sort spawners according to the levels of soluble R *salmoninarum* antigen in the parent fish. Under the guidance of Fish Health Biologist Steve Leek and Hatchery Manager Gary White, the Service pioneered BKD-segregation procedures at the Warm Springs NFH. This work began in the mid-1980's with FAT procedures and evolved into the use of ELISA technology when those methods were first developed at the Seattle National Fishery Research Center. Many refinements in ELISA-BKD segregation were achieved at the Dworshak FHC during cooperative deployment of this technology that involved personnel from the Dworshak NFH, Idaho FRO, and the Seattle NFRC. These advancements reinforced increases in the understanding of the epizootiology of BKD resulting from this Project,

Concomitant advancements were also made in understanding bacterial coldwater disease (BCWD) through systematic monitoring and through histopathological studies on infected juvenile fish. Most cases of BCWD were diagnosed in cultured coho salmon (80.9% of Service cases of BCWD) but, other species such as chinook (13.9%), and steelhead trout (5.2%) were also impacted. BCWD is now recognized as needing further management effort and research emphasis.

Of the four bacterial diseases covered by the Project at Service facilities, mortality-associated cases of BKD were the most common (58.6%), followed by BCWD (31.0%), ERM (6.1%), and furunculosis (4.3%).

Parasitic pathogen monitoring - The parasitic diseases of salmonids included in the Project were whirling disease, caused by *Myxobolus cerebralis*, ceratomyxosis, caused by

Ceratomyxa Shasta, and proliferative kidney disease (PKD), caused by the PKX organism which is believed to be an unidentified myxosporidean. The existing pre-Project Service fish health program fully covered Project requirements for sampling and screening for whirling disease. Both adult and juvenile salmonids were monitored for ceratomyxosis and PKD during the Project. Light infections of ***M. cerebralis*** were found in adult summer steelhead trout straying into the Warm Springs NFH on their up-stream migration back to the Umatilla (Northeast Oregon) River. No strays were passed above the hatchery and whirling disease has not been detected in fish reared at any of the Columbia River basin NFH's.

C. Shasta infections were frequently found in the intestinal tracts of spawning adult salmon at NFH's throughout the Columbia River basin. In no case could ceratomyxosis be deemed to be the sole cause of pre-spawning mortality but the ***C. Shasta*** infections should be regarded as complicating infections associated with terminal cases of funiculosis, ERM, or BKD. No evidence of PKD was detected in adult or juvenile fish at NFH's located in the Columbia River basin.

Organosomatic indices data collection - Organosomatic indices are numerical scores accumulated during detailed necropsy examinations of numerous organs and tissues collected from a random sampling of smolts just prior to their release for down-stream migration to the sea. Two Service-reared index stocks were included in the Project. Approximately 10 percent of the Dworshak NFH summer steelhead and the Spring Creek NFH tule fall chinook are marked annually with coded-wire tags for harvest management and fishery management purposes. Organosomatic indices data was collected annually from 60-fish samples from each release group of these two fish stocks.

Little is known of the predictive value of organosomatic indices monitoring as no provisions were included in the Project to relate adult survival or contribution of index (marked) stocks to the organosomatic indices data that was collected before the fish

were released as smolts. Finally, organosomatic indices data was of little value in predicting or diagnosing disease problems. It was noted that when infectious diseases occurred, typical disease signs usually were quite obvious before consistent changes in organosomatic indices were seen. While the value of organosomatic indices data may have limited immediate or “real time” value, the process caused close and detailed examination of the internal organs of thousands of fish which was educational to the personnel doing the work. Although the real value of monitoring organosomatic indices may have been missed by not collecting and analyzing corresponding adult return or contribution data, the labor costs and the necessity to kill large numbers of fish simply for data collection may make this work more costly than the benefits derived, except in specific research situations.

Histopathological services - Prior to the start of the Project, histopathological services supporting fish disease diagnoses in the Pacific Northwest were of limited availability from research institutions where such services conflicted with on-going research activities. The Project provided the Service with a portion of the funding necessary to support a full-time histopathologist and a technician and provided the necessary equipment and supplies to provide services to the fisheries agencies participating in the Project. Over 10,000 individual samples were prepared and examined histologically by John Morrison and the staff at the Olympia Fish Health Center. The Service will continue to provide histology services after the Project is completed.

Hatchery water supply data collection - During the first year of the Project, the Service submitted, in collaboration with the steering committee, a “Water Sampling Plan for Fish Health Monitoring” to meet contract requirements as provided in Task 4.1. The plan, submitted in final form on November 17, 1987, identified a total of 32 ground water sources and 11 surface water sources at the 13 Columbia River basin NFH's. Provisions in the plan covered temperature, dissolved gasses, chemical parameters to monitor, and set forth a schedule for collecting samples. BPA was to solicit bid quotations from analytical laboratories and negotiate sample collection and shipping protocols. Due to

the lack of guidance from BPA or their authorization for funds expenditure, further work called for in Objective 4.2 to “collect designated water samples twice annually from each hatchery water supply during the period of seasonal high flow and [seasonal] low flow [and] to ship water samples to the BPA-designated location(s) for chemical analysis” was not completed. Data called for in Objective 4.3 on flow indexes and loading densities was recorded and reported with case history reports.

Data collection and database management - In the early 1980’s the Service developed a comprehensive computerized Fishery Resource Evaluation Database (FRED) system that included a “stand-alone” section for storing and retrieving detailed fish disease case histories. This system utilized dBase III computer software. It covers a long list of data fields from which the 30 items covered by the Project for “additional data required in every anadromous fish health monitoring report” were selected. The Service was utilizing this system before the Project began and will continue to use it after Project completion.

Considerable data was requested under Objective 6.0 for “estimating the Project’s Benefits.” Paper records from Service files and pre-Project data from the FRED fish health database were used to produce the pre-Project case history data for 1983, 1984, and 1985 requested in Objective 6.1. Other data, requested in Objectives 6.1.1 through 6.1.5, for the pre-Project years of 1983 - 1985 simply was not consistently available for all Columbia River basin facilities or fish stocks in a form suitable for analysis and reporting in the manner requested. At the time the Project was being initially planned (1984 - 1986) computers and computerized fish health data systems in Service field stations were not in common use. This Project enabled the conservation agencies to make major advancements in the storage, analysis, and reporting of data but the collection and reporting of data from the pre-Project era is difficult and laborious and in many cases the existing data does not match up well with the database designed for the Project.

Conclusions

Benefits derived from the project - Service participation in the Project has been productive since the outset five years ago. Improved fish pathogen surveillance, enhanced interagency communications, similar computerized databases, histopathology services, and the development of parity among the fisheries agencies in their ability to provide state-of-the-art diagnostic services in the field are obvious benefits that will remain. In addition, applications of new technology have helped all participants improve the health, quality and performance of hatchery-reared fish. With BPA assistance the Pacific Northwest fisheries agencies have provided useful new information and insights of great value to fishery programs throughout North America and around the world. Specific Project benefits to the Service include:

- The development of the technology and methodology to collect, process, assay, record, and make use of data from thousands of individual fish annually to aid in the containment of fish pathogens while enabling the safe transfer of eggs or fish to other facilities or watersheds with minimal risk of spreading important fish diseases;
- The adoption of technology developed under this Project outside of the Columbia River basin which may have contributed to the early detection of VHS virus in Northwestern Washington, in 1988, before the virus became established in cultured fish;
- The incorporation of monthly station visits by fish pathologist, as the norm, has improved communications, increased pathogen surveillance, and has likely led to earlier disease detection and control resulting in reduced fish losses;
- The establishment of a high quality, high capacity histopathology support capability at the Olympia Fish Health Center aids in accurate disease diagnosis and in understanding the physiological changes that take place in diseased fish;

- The development of a reliable fish health database system supporting the storage and analysis of the information obtained during the collection, processing and analysis of a large number of samples taken from adult or juvenile salmon and steelhead;
- A multi-agency determination of which field services, which fish health monitoring strategies, and what technology will pay the best post-Project dividends in improved fish health and quality; and,
- A long list of facility impediments which, if rectified, likely would, themselves, prevent many of the disease problems that continue to persist.

Project elements to be sustained by the Service after contract termination - Some activities and capabilities, made possible through this Project, that will be continued as a part of the routine Service fish health monitoring program after Project completion include:

- Individual sampling of adult salmon or steelhead of some fish species at some locations, but sampling of adults for EIBS and listed fish parasites will be curtailed;
- Continued full-scale operation of the histopathology support services at the Olympia Fish Health Center;
- Continued use and improvement of an inter-active database system for storing and analyzing large amounts of fish health data; and,
- Continued interagency communications and collaboration to exchange information on the occurrence of fish diseases, procedures for disease prevention and control, and on advancements in technology.

Post-Project fish health needs remaining - The multi-party planning group that initially agreed upon the scope and content of the several Objectives composing this Project was very insightful in most respects. There were, however, some Project elements left undone including:

- Reducing or eliminating some of the important “impediments to fish health”, identified under Objective 2.3. This work would largely entail capital improvements in pathogen-free water supplies and improved broodstock holding and juvenile rearing facilities, but these and other improvements could make significant, broad-spectrum contributions to the immediate improvement of fish health and quality;
- Improvements in technology transfer to fish cultural programs in the form of “best management practices” to prevent or control fish diseases; and,
- Continued efforts to reduce the severe, long-term, chronic impacts of BKD and BCWD on susceptible fish species.
- The completion of the Project disbands an important inter-agency link through which research needs could be identified and prioritized in the context of current agency programs and policies and in direct support of the Council’s Fish and Wildlife Program objectives.

The completion of this five-year contract ends a significant amount of funding for the Service’s fish health program. It is not something that is occurring without warning, however. Service planning for the support of essential elements of the Project began more than three years ago. There remains, however, much data evaluation and analytical work to glean useful information from the vast amount of data recorded. It is unknown if definitive answers to long-nagging questions on smolt-to-adult survival lie within the large accumulation of data. Consultation and collaboration, through well developed communications channels, with the other parties participating in the Project may yet yield new approaches to solving old problems.

Acknowledgements

The patient guidance and administrative assistance provided by Ron Morinaka, BPA Project Manager, is appreciated. He has worked effectively to clarify contract obligations, convene and moderate Technical Steering Committee meetings, and has steadfastly facilitated Service efforts to meet requirements.

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